

EFFECT OF SCHEDULE FEEDING ON WATER INTAKE AND URINE OUTPUT IN RATS

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Abstract : Water intake of schedule feeding rats was correlated to food intake through variations in calorie content of food. On intake time restriction (3h) schedule, it was positively correlated while on amount restriction schedule (25% and 15% food) correlation was negative. Water-to-food ratio (W/F) of 3hFW rats was decreased whereas W/F of 25% and 15% food animals, it was increased as compared to *ad lib* W/F. On calorically rich (3.2 cal/gr) diet 3hFW rats food intake (7.8 ± 0.6 gr) and water intake (4.7 ± 0.3 ml) remained unaltered, while *ad lib* rats food intake (14.7 ± 0.9) was decreased and water intake (16.2 ± 1.1) increased as compared to their intake on calorically poor (2.8 cal/gr) diet.

Urine percent over water intake ($u/w \times 100$) was inversely related to food intake of rats (on *ad lib* food, 13.8%; on 25% food 29.1% on 15% food 31.8%) excepting for urine percent of 3hFW rats which was (7.6%) disproportionately decreased.

Key words : water intake schedule feeding urine output

INTRODUCTION

There are several kinds of drinking which may have different constellations of determinants (1). Among them meal associated drinking is important as it accounts for nearly 7/8 of total water intake per day (2). Solid food intake produces post absorptively intravascular hyperosmolarity which acting on chemoreceptors located in preoptic area initiate drinking behaviour while its stimulation of receptors in anteroventral wall of third ventricle (AV3V) promotes antidiuresis (3). The osmolar effects on thirst and urine output are known to be independent (4) and differ in their stimulus thresholds. AV3V osmoreceptors appear to have a considerably lower stimulus threshold than those receptors subserving elicitation of urge to drink (5).

Reviewing food intake related drinking, Fitzsimons (6) expressed nearly 24 years ago that under *ad lib* and stable conditions "there is a highly significant positive correlation between the amount of water drunk along with the meal and the size of that meal" which has been amply proved by later investigations (7) with slight variations in nocturnal and diurnal food intake associated drinking (8, 9). In contrast to robust relation between water-food relation under *ad lib* conditions the water-to-food relation under schedule feeding appears to be not so clearly established. Some have reported polydipsia and polyuria (10, 11) and others observed hypodipsia and oliguria (12, 13). The above contradictions may be due to type of feeding schedule used. Hence two types of feeding schedules, namely limited food intake regimen and restricted feeding time schedule are used in this

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investigation. As these two types of feeding schedules are known to cause calorie intake reduction (7, 14) and since it is not clear whether calorie intake reduction *per se* has any effect on water intake, it is necessary to delineate that aspect also. The present investigation is therefore aimed at identifying the effects of schedule feeding on water intake and urine output of rats fed with calorically varying diets.

METHODS

Adult (3 months old) male rats, housed in individual cages were used. They were kept in animal house temperature of which was maintained at $24^{\circ}\text{C} \pm 2.0$. The light-dark changes in room followed natural diurnal alternations. Animals were adapted to cages, food and water intake for 10 day period before use as experimental animals. The cage had wire mesh bottom which allowed faeces and urine to drop out of the cage. Two varieties of wet, mashed diet were prepared using stock food powder (4.2 cal/gr) and water as was done earlier (13). One food mixture contained stock powder and water in 3:2 ratio and the other in 5:1 ratio. The first more dilute mixture was calorically poor (2.5 cal/gr) food, while the other was comparatively rich in calories (3.5 cal/gm). Tap water in a measuring cylinder fitted with a spout was available for drinking.

The following groups of rats were used:

- Group 1: *Ad lib*, which had food and water all the time.
- Group 2: 3 h food and water (3 hFW). Food and water were available only for 3 h/day. The group was adapted to schedule for 8 day period before the investigations were initiated.
- Group 3: 25% diet. 25% of their *ad lib* food intake was allowed. Water was

available all the 24 hrs. The group was adapted to schedule for 20 day period, prior to investigations.

- Group 4: 15% diet. 15% of *ad lib* food intake was given. Water was available all the time. Adaptation period (20 days) was similar to that of 25% diet group.
- Group 5: Starved. Food was withheld for 7 days but water was available *ad lib*.
- Group 6: Thirsty. Food was given *ad lib*. Water was not given for 7 days period.

The ratio allotted to any one group remained in it till the end of investigation.

Measured amounts of fresh food and water were replenished daily at about 4 p.m. One hr before food the animals were weighed, their food and water intake of previous day was measured. The animals were fed with dilute diet for a period of 7 days followed by another period of 7 days on concentrated diet. The starved and thirsty groups were used only during the period when dilute diet was given. Measurements of body weight, food and water intake on calorically diluted as also on dense food were correct to 0.2 g or 0.2 ml. Intake is expressed as per 100 g body weight.

Investigations of urine output were conducted on all groups or rats only during the period when they were fed with dilute diet. For collecting urine, funnels closely fitted to the wire mesh bottom were used. Urine measurements are correct to 0.1 ml and expressed as per 100 gr body weight.

At the end of experiment six animals each from *ad lib*, 3hFW and 25% diet group were sacrificed by decapitation. The heart, liver, stomach intestine and gastrocnemius muscle

were isolated and their wet weight taken. The tissues were then transferred to hot air oven kept at 80°C for drying. Tissues were periodically weighed till the dry weight was consistent. The % of water in the tissues was then computed from wet and dry weight.

Students' 't' test and correlation coefficient were used for statistical analysis of data. $P < 0.05$ is taken as significant level.

RESULTS

Table I shows W/F ratio and water-to-food correlations on calorically poor food. The food intake of 3 hFW rates (8.5 ± 0.18) and of rats on 25% diet (4.7 ± 0.08) and 15% diet (2.8 ± 0.02) were significantly reduced as compared to *ad lib* food intake (17.5 ± 0.36). Likewise the water intake of all experimental groups was less than *ad lib* water intake and correlated with food intake ($r=0.4$ to 0.6). However, while water intake of *ad lib* and 3 hFW was positively correlated, the water intake of 25% and 15%

diet groups was negatively correlated with their food intake. All the correlations were significant ($P < 0.045$ to 0.01). The computation of W/F showed that 3hFW animals W/F (0.6 ± 0.0002) was approximately comparable to *ad lib* rats W/F (0.7 ± 0.0002). The W/F of 15% diet group (1.2 ± 0.07) and the W/F of 25% group (0.9 ± 0.5) were increased over *ad lib* rats W/F.

Table II shows intake on calorically rich food. It may be that W/F ratios of *ad lib* and semi starved rats are increased over their W/F on calorically poor food, while W/F of 3 hFW rats remained unaltered. Water-to-food correlations of all groups of rats remained approximately similar to correlations shown on dilute diet.

The investigations of urine output showed (Table III) that the volume of urine excreted per day/100 g body weight was decreased in 3 hFW (0.57 ± 0.05) as well as in water deprived (0.1 ± 0.07) and semi starved animals (25% group 1.3 ± 0.08 , 15% group 1.1 ± 0.06) in comparison to urine excreted by *ad lib* rats (2.3 ± 0.08).

TABLE I : Mean (\pm SE) food (gr) and water (ml) intake per 100 gr body weight of rats on calorically-poor diet.

Group	Food	Water	Water / Food	Water-Food correlation
1. <i>Ad lib</i> (25)*	17.5 ± 0.36	12.4 ± 0.33	0.7 ± 0.002	+0.5
2. 3 hFW (10)*	8.5 ± 0.18	4.9 ± 0.19	0.6 ± 0.002	+0.6
3. 25% diet (26)*	4.7 ± 0.08	4.2 ± 0.13	0.9 ± 0.05	-0.4
4. 15% diet (26)*	2.8 ± 0.02	3.4 ± 0.12	1.2 ± 0.07	-0.5
5. Starved (6)*	-	5.6 ± 0.75	-	-
6. Water deprived (6)*	8.6 ± 0.18	-	-	-

*Number in parenthesis indicates the number of rats in group.

TABLE II : Mean (\pm SE) food (gr) and water (ml) intake per 100 gr body weight of rats on calorically rich diet.

Group	Food	Water	Water / Food	Water-Food correlation
1. <i>Ad lib</i> (25)*	14.7 ± 0.9	16.2 ± 1.1	1.1 ± 0.08	+0.6
2. 3hFW (10)*	7.8 ± 0.6	4.7 ± 0.3	0.6 ± 0.04	+0.5
3. 25% food (26)*	3.7 ± 0.03	9.9 ± 0.6	2.7 ± 0.57	-0.5
4. 15% food (26)*	2.2 ± 0.08	7.3 ± 0.6	3.2 ± 0.4	-0.7

*Number in parenthesis indicates the number of rats in group.

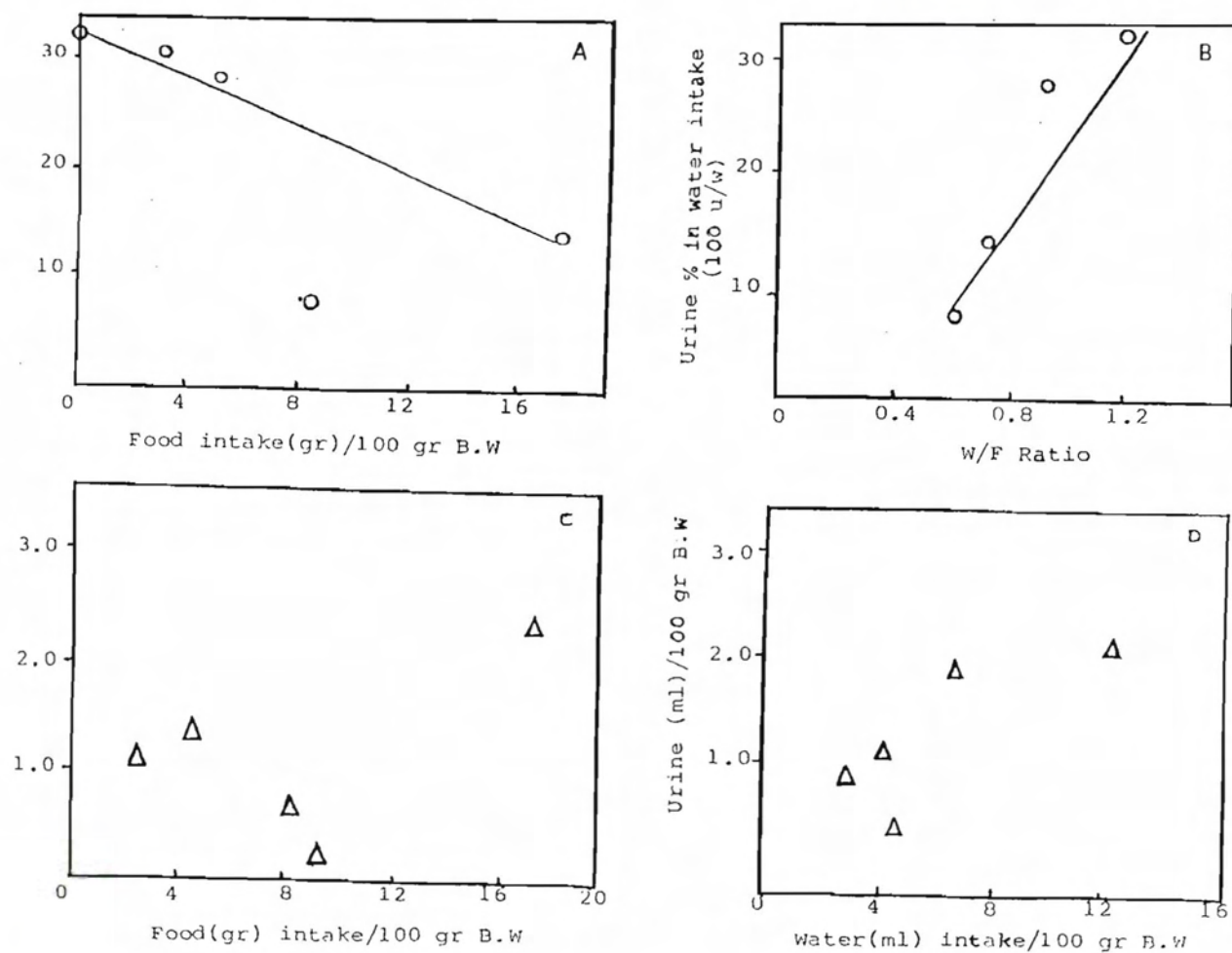


Fig. 1: Relation of food intake (Panel A), and W/F ratio (Panel B) to urine % in water intake. Panel C and D show that urine output (Volume) is unrelated to either food intake or water intake.

TABLE III: Mean (\pm SE) ml of water intake and urine output/100 gr body weight of rats on calorically poor diet.

Group	Water	Urine	$\frac{\text{Urine}}{\text{Water}} \times 100$
1. Ad lib	12.4 \pm 0.33	23 \pm 0.06	13.8 \pm 0.43
2. 3 hFW	4.9 \pm 0.19	0.57 \pm 0.05	7.6 \pm 0.67
3. 25% diet	4.2 \pm 0.13	1.3 \pm 0.08	29.1 \pm 2.8
4. 15% diet	3.4 \pm 0.12	1.1 \pm 0.04	31.8 \pm 3.3
5. Starved	5.6 \pm 0.75	1.97 \pm 0.4	32.8 \pm 4.56
6. Water deprived	-	0.1 \pm 0.07	-

However, the urine output of totally starved rats (1.97 ± 0.4) was almost similar to *ad lib* rats urine output.

The variations in volume of excreted urine were not related to either water intake or food intake of respective groups of rats (Fig. 1-C and D). DO. However, the % of urine in water intake ($U/W \times 100$) was inversely related to food intake (Fig. 1A) but not to water intake (not shown). It was also approximately linearly related to W/F (Fig. 1-B). It is noteworthy that % urine fraction of 3hFW group was significantly less (i.e. renal retention was more) than that expected from its food intake (Fig. 1-A).

Despite absence of relation between water intake and urine output the water content of tissues investigated was approximately similar (74-75%) thus indicating that tissue water content was defended under varying conditions of water intake.

DISCUSSION

Rats were used in this investigation as it facilitates comparison of our results with extensive data already available on their water intake (5, 15). Male rats alone were used here as their daily food and water intake are more stable as compared to intake of female rats (16). Since the rats become active at about 4 pm, the food and water were replenished at 4 pm for optimisation of intake and facilitation of adaptation to 3 h food and water schedule (17).

The positive correlation of water-to-food intake of *ad lib* rats and 3 hFW animals reinforced earlier reports (6) in rats (13, 18) in pygmy goats (19) and in pigs (20). It is possible that the post-absorptive hyperosmolarity of body fluids was responsible for positive correlation shown in *ad lib* rats intake, as food and water were freely available to them all the time. But

the correlation shown in 3hFW rats is unlikely to be due to post absorptive clues, because during the time taken for digestion and absorption of the final meal and also later (roughly 21 hrs), the water was not available for drinking. Hence positive correlation of water to food intake of 3 hFW rats ought to be due to pre-absorptive cues (21). The pregastric food contingent set of signals (bulk, texture, mastication, deglutition) are known to initiate mealtime drinking in the absence of systemic dehydration (22), while other preabsorptive cues (gastric, intestinal and hypovolemic) sustain drinking till needed amount of water is drunk (23). For 3 hFW rats the preabsorptive hypovolemia resulting from sequestration of interstitial water into GI tract (24) in proportion to unusual amount of food 'crammed' into stomach might have provided cues for drinking. In contrast to *ad lib* and 3 hFW rats the semi starved rats showed a negative correlation of water to food intake which was further supported by the water intake of totally starved rats. It may be noted that these animals on food deprivation schedule also reduced water intake accordingly as was noticed earlier by Kutscher (12) but the reduction in water intake was inversely related to reduction in food intake. Excess water ingested than necessitated by solid food intake was subsequently excreted via urine and not retained in any tissue as evidenced by computation of tissue water %. Why do the starved and semi starved rats drink excessively? Probably drinking provides a transient relief from painful hunger pangs and even cause a sense of satiation via stomach distension (24, 25).

Food and water intake on two food mixtures evidenced that intake regulation in 3hFW rats is different from that of *ad lib* rats. The *ad lib* rats intake is calorically regulated as evidenced by increased intake on calorically poor diet as compared to the intake on calorically-rich food. It confirmed earlier evidences (26, 27). Further

the total daily water intake (from spout plus water in food mixtures) of *ad lib* rats on dilute food was approximately similar to total water intake on concentrated food. The constancy of water intake was achieved by adjustment of intake via spout. Thus on dilute food (containing more water) the water intake was reduced as compared to spout water intake on denser food. Even the food restricted (25% and 15%) animals which had *ad lib* water evidenced reduced water intake on calorically diluted diet. In contrast 3hFW rats apparently in a hurry to eat drink ingested similar amounts (grams) both on calorically rich food as well as on calorically less dense food disregarding even the fast acting gustatory and gastric signalisation of calories (28, 29). Even their water intake was similar on both types of food. It therefore appears that 3 hFW rats intake is regulated on the basis of volume (bulk).

The urine output of 3 hFW rats was surprising. As their W/F (0.6 ± 0.002) was almost similar to *ad lib* rats W/F (0.7 ± 0.0002), the urine expressed as % of water intake ought to be also similar to *ad lib* rats $u/w \times 100$. But 3 hFW rats $u\%$ was approximately half of *ad lib* $u\%$. The conserved water was not stored in tissues, as tissue water% of 3 hFW and *ad lib* rats were comparable. As the body weight of mealtime rats is known to increase slowly (13, 17) after the initial loss following meal-time restriction, it is presumed that the conserved water was used for tissue building. The enhanced renal conservation of water in 3 hFW animals ought to be due to increased ADH release, in

response to large meal (7.8 to 8.5 gr) stuffed into stomach in 3 hrs. Hypovolemia/hyperosmolarity consequent to gastric gorging could have initiated thirst and ADH release from two distinctly different brain areas which are functionally independent of one another (4). Thus enough of water was ingested to quench the thirst and keep normal W/F ratio, while the ADH acting independently of thirst conserved water. In contrast *ad lib* rats eat small meals (1.6-1.7 gr) more frequently (16), the hypovolemia (or hyperosmolarity) of interstitial fluid following such small meals may not be adequate to cause antidiuresis. The ADH activity appears to be much reduced in starved and semi-starved rats as indicated by increase in urine % of water intake. On scanty amount of food or on total deprivation of food a large portion of ingested (29-33%) water was unused and hence excreted.

The rapid adaptation (in 7 days) of thirsty rats to water scarcity and *ad lib* food condition was evidenced by drastic decrease in urine output (0.1 ± 0.007 ml/day). Additionally, despite absence of water the intake on food (8.6 ± 0.18 gm) in 24 hrs, was similar to 3 hr intake of meal-time rats which had access to water to wet their oral cavity during eating. Though food intake was similar, the thirsty rats lost more body weight (30-35%) in 7 day period following initiation of schedule, than the loss incurred by meal-time rats (20-25%). It probably indicates the importance of water in defence of body weight (13).

REFERENCES

1. Epstein AN. Consensus, controversies and curiosities. *Fed Proc* 1978; 37: 2711-2716.
2. De Castro JM. A Micro regulatory analysis of spontaneous fluid intake by humans: Evidence that amount of liquid ingested and its timing is mainly governed by feeding. *Physiol Behav* 1988; 43: 705-714.
3. Buggy J, Hoffman WE, Philips MS, Fisher AE, Johnson AK. Osmosensitivity of rat third ventricle and interaction with angiotensin. *Am J Physiol* 1979; 5(1); R 75-82.
4. Peck JW, Blass EM. Localisation of thirst and antidiuretic osmoreceptors by intracranial injections in rats. *Am J Physiol* 1975; 228 (5): 1501-1509.

5. Anderson B. Regulation of water intake. *Physiol Rev* 1978; 58: 582-603.
6. Fitzsimons JT. Thirst. *Physiol Rev* 1972; 52: 468-561.
7. Rao BS, Shekar Babu H. Effects of intake-time restrictions on behavioural intake. In: studies in animal behaviour (Ed) Rao BS and Shetty PS. Suppl. Bulletin. Ethological Society of India III, 1984; 23-28.
8. Bealer SL, Johnson AK. Preoptic hypothalamic periventricular lesions alter food associated drinking and circadian rhythms. *J Comp Physiol Psychol* 1980; 94: 547-555.
9. Johnson RR, Johnson AK. Light/dark cycle modulates food-to-water intake ratios in rats. *Physiol Behav* 1990; 48: 707-711.
10. Crow LF. Subcommissural organ, later hypothalamus and dorsal longitudinal fasciculus in water and salt metabolism. In: Thirst (Ed) MJ Wayner, Pergamon Oxford, England, 1964.
11. Falk JL. Conditions producing Psychogenic polydipsia in animals. *Am NY Acad Sci* 1969; 157: 569-593.
12. Kutscher CL. Species differences in the interaction of feeding and drinking. *Am NY Acad Sci* 1969; 157: 539-552.
13. Rao BS, Sharma KN. Effects of disruption of diurnal rhythms in food intake of rats. *Indian J Physiol Pharmacol* 1980; 24: 170-176.
14. Rao BS. Effects of dynamic and static body weight loss on taste responses. *Indian J Physiol Pharmacol* 1983; 27: 25-31.
15. Kraly FS. Physiology of drinking elicited by eating. *Psychol Rev* 1984; 91: 478-490.
16. Wade GN. Gonadal hormones and behavioural regulation of body weight. *Physiol Behav* 1972; 8: 523-534.
17. Rao BS, Thomas A, Prabhu VN. Effects of meal-time restriction on water intake in rats. *Ind J Exptl Biol* 1984; 22: 439-441.
18. Glendinning JI, Smith JC. Consistency of meal pattern in lab rats. *Physiol Behav* 1994; 56(1): 7-16.
19. Rossi R, Scharrer E. Circadian patterns of drinking and eating in pygmy goats. *Physiol Behav* 1992; 51: 895-897.
20. Houpt TR. Gastric pressures in pigs during eating and drinking. *Physiol Behav* 1994; 56: 311-317.
21. Lepkovsky S, Lyman R, Fleming D, Nagumo MD, Dimmick MM. Gastro intestinal regulation of water and its effect on food intake and rate of digestion. *Am J Physiol* 1957; 188: 327-331.
22. Kraly FS. Pregastric food - contingent stimulation elicits drinking in the absence of systemic dehydration in rat. *Physiol Behav* 1990; 48: 841-844.
23. Kraly FS. Drinking elicited by eating In: Progress in Psychology, Vol. 14 (Ed) Epstein and A. Morrison, Academic press, New York, 1990.
24. Paintal AS. A study of gastric stretch receptors: Their role in peripheral mechanism of satiation of hunger and thirst. *J Physiol* 1954; 126: 255-270.
25. Sharma KN, Anand BK, Dua S, Singh B. Role of stomach in regulation of activities of hypothalamic feeding centres. *Am J Physiol* 1961; 201: 593-598.
26. Jacobs HL, Sharma KN. Taste Versus Calories : Sensory and metabolic signals in the control of food intake. *Am NY Acad Sci* 1969; 157: 1084-1125.
27. Prabhakar E, Rao BS. Rapid caloric metering in *ad lib* rats. *J Biosci* 1985; 9: 41-45.
28. Prabhakar E, Rao BS. Effects of gustation and gastric loading on rapid calorie metering in rats. *Physiol Behav* 1989; 45: 685-688.
29. Rao BS, Prabhakar E. Effects of body weight loss and taste on VMH-LH electrical activity of rats. *Physiol Behav* 1992; 52: 1187-1192.